

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of  
Antoni BANAS et al  
Serial No. 09/709,457  
Filed November 13, 2000

GROUP 1638  
Examiner R. Kallis

USE OF CLASS ENZYMES AND THEIR ENCODING GENES TO INCREASE THE  
OIL CONTENT IN TRANSGENIC ORGANISMS

**DECLARATION UNDER 37 CFR §1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Dr. Oliver OSWALD, hereby declare as follows:

I am a scientist at BASF Plant Science GmbH in Germany. My relevant background and experience are set forth on the attached Curriculum Vitae. I make this declaration in support that one of ordinary skill in the art would be able to make and use the claimed invention.

As evidence of this assertion, enclosed are results of a study analysing the triacylglycerol content (TAG) in the seed of *Brassica napus* plants that over express the ARE1 gene of *Saccharomyces cerevisiae*. The study shows that *Brassica napus* plants that over express the yeast ARE1 gene exhibit a significant increase in oil content in the seed. These studies have been conducted in view of the present application and techniques known to a person working in the area of plant genetics.

The study may be characterized as follows:

*Brassica napus* lines expressing the yeast ARE1 gene from a napin promotor were generated by *Agrobacterium* transformation (Bna 5812 7/3 and Bna 5812 1/1). The oil content in T1 seeds from transformed lines with confirmed yeast ARE1 expression were analysed and compared to seed oil content in control plants (Bna 49104) transformed with an empty vector.

The extraction of lipids from the seeds was performed according to Bligh & Dyer (1959) Can J Biochem Physiol 37:911. The seed material was broken down by the use of a ball mill (MM300 of company Retsch (Haan)). The solvent used was Chloroform/Methanol (2:1; containing Mono-C17-glycerol from Sigma as internal standard). After addition of potassium phosphate buffer having a pH of 7.5, phase separation was initiated. As to the organic phase, an aliquot was taken, diluted with chloroform, and spotted onto the capillary Chromarods SIII of the company Jatroscan (SES, Bechenheim). A two-step separation was performed by thin layer chromatography in 6:2:2 Chloroform: Methanol: Toluol followed by 7:3 n-Hexane:Diethylether as a solvent mix. The samples were measured using a Jatroscan MK-5 (SES, Bechenheim) according to Fraser & Taggart, 1988 J. Chromatogr. 439:404. Quantification was performed using the internal standard and calibration curves with Tri-C17-glycerin (Sigma) and the software ChromStar (SES, Bechenheim).

The amount of TAG measured was standardised on the weight of the seed material used for the extraction, and the percentage (on a weight basis) of the seed material consisting of TAG calculated. The results are shown in the graph enclosed in this declaration. Since over 98 % (w/w) of seed oils typically consist of TAG (see the enclosed table 9 on page 3 summarizing the nontriglyceride components of canola oil, taken from 'Introduction to the processing of fats and oils' AOCS press, available in parts at <http://www.aocs.org/press/mod3sample.pdf>; see also 'Introduction to fats and oils technology', p25, Editors R.D. O'Brien, W.E. Farr & P.J. Wan, AOCS Press, ISBN 1-893997-13-8) a measurement of TAG content rather than total fatty acid content (originating from seed oil and membranes) of the seeds is an even better measurement of seed oil content. An increase of TAG as shown results therefore in an increase in seed oil from *Brassica napus* seeds.

TABLE 9 from "Introduction to the processing of fats and oils"

Nontriglyceride Components of Crude, Water Degummed and Chemically Degummed, Canola Oil

	Crude oil	Water degummed	Chemically (acid) degummed
Free fatty acids, %	0.4-1,2	0.4-1,2	0.4-1,2
Phosphorus, mg/kg <sup>a</sup>	300-500	100-250	5-50
Sulfur, mg/kg <sup>b</sup>	3-15	3-15	3-15
Chlorophyll, mg/kg <sup>c</sup>	5-35	5-35	5-35
Fe, mg/kg <sup>d</sup>	2-5	2-5	<0,5

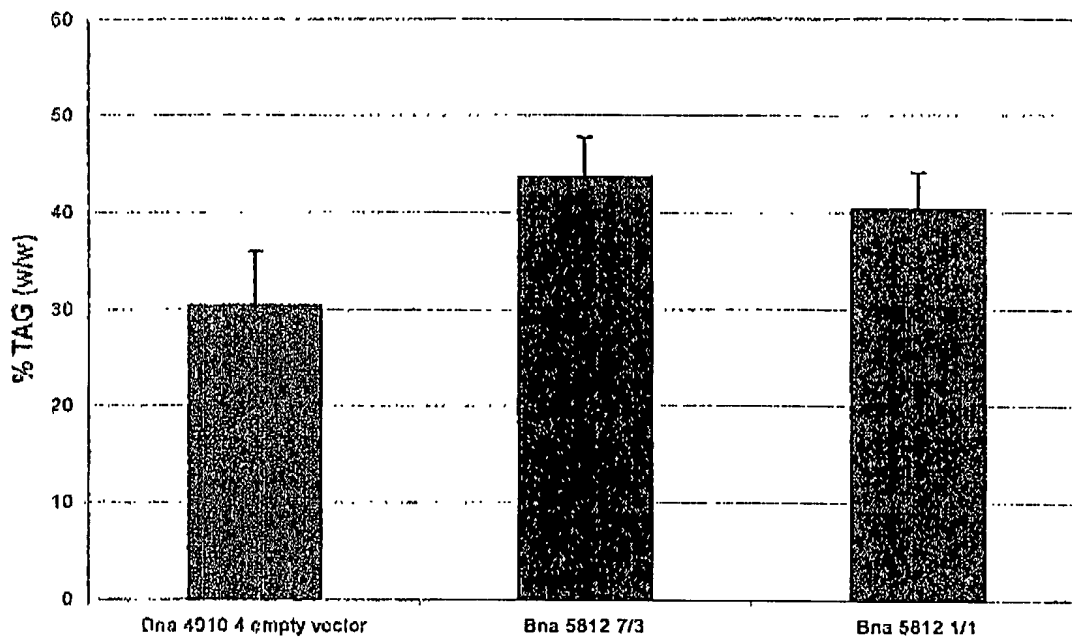
<sup>a</sup> Degumming affects only the phosphorus content of the oil.

<sup>b</sup> Sulfur, if present, is not decreased, as in rapeseed.

<sup>c</sup> Chlorophyll content is not decreased.

<sup>d</sup> Iron content is usually removed to only a minor extent, except in chemical degumming.

*Brassica napus* seed from plants over expressing the yeast ARE1 gene (identified as line Bna 5812 7/3 and 5812 1/1 show a significant increase in oil content (more than 10% increase in TAG) as compared to seed from plants that do not over express ARE1 (identified as Bna 4910 4 empty vector control). For the line 5812 7/3:  $p = 0,026299756$ . The results are shown in the figure below. The results are based on the analysis of 10 individual transgenic plants per line with 3 separate extractions of seed per plant.



The results from Brassica napus demonstrate that the disclosure of the patent application is enabling for a general method for increasing the oil content in seeds of an oil producing plant as claimed.

The undersigned declares further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

14. 12. 2003

Dr. Oliver OSWALD

*O. Oswald*

## CURRICULUM VITAE

I, Oliver Oswald, holding a PhD in plant biochemistry, a citizen of the Federal Republic of Germany and residing at Bexbacher Str. 5, D-67063 Ludwigshafen, Germany, declare as follows:

I am a fully trained biologist, having studied biology at the University of Mainz from 1993 to 1995 and the University of Glasgow from 1995 to 1997;

I obtained my doctor's degree from the University of Glasgow in 2001:

Since 2001, when I joined BASF Plant Science GmbH, Germany, I have been working in the field of plant molecular biology and plant genetic engineering;

I am not one of the inventors of the invention disclosed and claimed in Application Ser. No. 09/709,457 but as I am working in the same research field I am familiar with the contents thereof;

I further declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.



Dr. Oliver Oswald

Signed at Ludwigshafen, Germany,  
This 4<sup>th</sup> day of December 2003